

Enoch

OFFICIALLY TESTED AT THE
PRUSSIAN INSTITUTE FOR THE INVESTIGA-
TION AND TESTING OF SERUM.

BY

GEH. MED. RATH PROF. DR. EHRLICH.

ANTI-DIPHTHERITIC SERUM.

(RUETE-ENOCH)

THE MODE OF ITS PREPARATION, TESTS,
AND ADMINISTRATION.

C. BISCHOFF & CO., 87 & 89 PARK PLACE,
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INTRODUCTORY.

In the following pages we have endeavored to describe the main features in the process of development of antidiphtheritic serum as carried on under the care of Drs. Ruete and Enoch. The usual dosage of Antidiphtheritic Serum (Ruete-Enoch) is as follows:

No. 0	(Yellow Label)	200 Units
No. I	(Green Label)	600 Units
No. II	(White Label)	1000 Units
No. III	(Red Label)	1500 Units

No. 0—200 units is used when it is desired to immunize, and this dose may be given those who have been exposed to infection.. Protection lasts apparently about three weeks.

This is also the usual dose in very mild cases, and when given early is sufficient.

No. 1—600 units is used in more marked cases, and by some physicians is preferred to the foregoing.

No. 2—1000 units is the usual dose required in most cases of diphtheria. As with any other strength, the injection should be made as early in the disease as possible, preferably within the first 24 hours.

No. 3—1500 units is intended for the most grave cases and usually causes marked improvement; in any case, however, and with any dosage, a second injection is given within twelve hours if the improvement is not decided. The indications will determine further repetition.

In addition to these, serum of higher potency is prepared also, but the efficacy of this stronger serum is by no means in harmony with the greater cost; more especially since it has been found that the strong serum loses its power and is unfit for use.

It is perhaps hardly necessary to say that each vial contains but one dose, no matter what the volume of the serum may be, or what its strength in units. The serum is intended only for use subcutaneously and all that vial holds should be used at one time. It is obvious that the serum must be used at once after the vial is opened and that it is, to say the least, most injudicious to use a serum that has been exposed to the air, which may mean infection.

Anti-diphtheritic Serum (Ruete-Enoch) is pure and its use is very rarely attended by any by-effects locally and none constitutionally. Great care as to asepsis is advisable in giving the injections. The syringe must be sterile and the skin at the site of injection also should be sterilized. The simplest and most approved method is to thoroughly cleanse the skin at a point where there is much subcutaneous connective tissue, scrubbing well with soap and water and using finally carbolic acid in 5 per cent. solution. The syringe also should be repeatedly washed out with this solution and, just before use, with sterile water. The injection is made as usual when medicaments are given hypodermatically. Inject slowly and quite deeply into the subcutaneous tissue.

The entire process of manufacture of our Anti-diphtheritic Serum (Ruete-Enoch) is under the control of the government and no serum can reach the public before it has been tested at the Prussian Institute for the Investigation and Testing of Serum at Berlin. Here it is re-tested under the supervision of Geh. Med. Rath Prof. Dr. Ehrlich, and must comply with the requirements of the government in every particular.

As evidence of satisfactory test, a lead seal is fastened to each vial by the government official. In this way the physician is always sure of obtaining serum suited for the treatment of diphtheria, since the lead seal guarantees a serum without faults. The uniform test to which each serum is subjected

at Berlin, is therefore an incalculable advantage. It enables us to use preparations not only without fault but of known and uniform strength. It is, in brief, an official standardization of the serum.

Proper sterilization of the syringe, as already stated, is of prime importance and we believe to this is due in part the uniformly good results obtained with the Anti-diphtheritic Serum (Ruete-Enoch). For, although this serum has been very extensively used, no complaint has yet been made of any objectionable effects whatever. In fact, physicians to whom the questions were put directly, stated that the serum was exceptionally well borne, readily absorbed and that not the least discomfort or pain at the site of the injection had ever been noted.

Another advantage in connection with the Anti-diphtheritic Serum (Ruete-Enoch) is that the entire process, from the production and testing of the toxin, the immunization of the horses up to the sealing of the vials—each individual manipulation, therefore, is carried out by Dr. Ruete and Dr. Enoch, personally; in this way the hands of laymen cannot possibly endanger the preparation, at any stage of its development.

Anyone can judge what labors and what scrupulous care this method entails, but for this very reason, all can absolutely depend upon the value of the preparation. This fact has rapidly smoothed the way for an extensive use of the serum, has secured its entrance into the large hospitals and has earned the esteem of the general practitioner. The latter has not only been spared much worry, but he has noted with pleasure the good results due to the favorable and *pure* action of the preparation, which must tend to give the practitioner a feeling of absolute certainty toward the public.

We would commend the preparation therefore to the careful consideration of the medical profession.

In urgent cases, and when it is impossible to obtain this serum from the local druggists, we will send it direct, if so desired.

In the following report of Dr. Enoch, we trust much will be found of interest regarding the method of preparing and testing the Anti-diphtheritic Serum (Ruete-Enoch).

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*THE METHODS OF PREPARING AND TEST- ING ANTI-DIPHTHERITIC SERUM,

By Dr. C. ENOCH, Hamburg.

Loeffler, in 1884, first recognized the constant occurrence of specific bacteria in diphtheria of man, and he succeeded in obtaining pure cultures from the pseudo-membrane formed in this disease.

The bacillus of diphtheria is rod-shaped, of about the size of the tuberculosis bacillus, is often slightly curved and frequently shows most peculiar evolutionary modifications. In stained preparations we usually find club-shaped nucleated bacteria. Although the bacteria are present in multitudes in diphtheria, the severe symptoms of the disease are due to the production of toxines (poisonous products), which enter the blood and cause such severe poisoning.

The bacillus is motionless and develops best at a temperature of 35°-40° C. (95°-104°F.). It does not liquefy gelatine and on this firm culture medium it forms small, globular colonies. For cultures of the diphtheria bacillus, various media have been tried, bouillon, blood serum, agar, etc., and it develops in varying degree on each. The diphtheria bacillus belongs to the strongly toxic germs and it forms in the cultures as well as in the body of a patient an unusually strong poison, which is diffused throughout the culture medium or throughout the patient's body. Of animals, the guinea pig is the most susceptible to this poison. On section there is found marked formation of cicatricial tissue at the point of injection, considerable pleural effusion and particularly reddening of the supra renal capsules. The diphtheria bacillus stains

*A paper read before the Society of German Chemists, at Hamburg.

well with anilin colors and is thus easily made visible in the specimen to be examined.

Having thus learned something of the properties of the diphtheria bacillus in these respects, let us now turn to the actual method of preparation of the curative serum. This process may be divided into three stages:

1. Production of the diphtheria toxin.
2. Immunization of the horses and abstraction of the blood.
3. Tests of the serum thus secured.

1. *Production of the Toxin.*—Simple as this may seem to be, just so difficult is it and just so dependent on the most various uncontrollable incidents. We have seen that during its growth in a culture medium, as bouillon for instance, the diphtheria bacillus imparts the poisonous products of its metabolism to the medium. On filtering out the bacteria from the bouillon, a diphtheria toxin solution is obtained free from germs. This same method we use, first cultivating great masses of the bacteria in bouillon in which they are allowed to develop in large ovens at a temperature of 37° C. (98. 6° F.). The bacteria gradually fall to the bottom and are removed by filtration. To the toxin thus obtained a small amount of toluol, tricresol or carbolic acid is added as a preservative. The time required for the proper development of the cultures is determined by tests and varies from 3-4 weeks depending on the form and size of the vessels used. Obviously, an accurate control is necessary over the absolute purity of the bacteria and for this purpose every culture is examined microscopically. All those which are impure or even seem to be so, are at once rejected. After filtration of the pure cultures we have before us the clear toxin solution, which now must be tested as to its strength or toxicity. In all diphtheria experiments or tests, the animals used are guinea pigs of middle weight, about

200-300 grams. In these animals various amounts of the toxin are injected subcutaneously, say from 0,1 gram downward. At first, the animals lose in weight and finally die when the fatal dose is reached. By means of further injections the minimum fatal dose is accurately determined and calculation is made for a guinea pig weighing 500 grams. The minimum fatal dose therefore is the least amount of toxin which will certainly kill a guinea pig weighing 500 grams within 4-6 days. Having determined this, the first step is completed: the production and test of the toxin. This product is best kept in a cool, dark place, and is retested from time to time. Let us assume that our minimum fatal dose is 0,1 gram. It need not be emphasized that in order to obtain the most potent toxin much depends on such minutiae as the temperature and duration of culture, very much too on the kind of bouillon, its alkalinity, the size of the culture glasses and even upon their shape. The consideration of these details now would however occupy too much time.

2. *Immunization of the Horses.*—Let us first consider what the stables must be and what requirements are demanded in the horses. The stables must be absolutely free from any fault whatever, they must be isolated, only the test animals must be admitted, the walls must permit of thorough disinfection, there must be a water-supply sufficient to maintain the most scrupulous cleanliness of the stable and of all utensils. There must be adequate room for each horse and sufficient air space, the stable must be well lighted and properly ventilated. The horses must be absolutely healthy and we require even more than that: The horses to be used for this purpose, must have been under the observation of a veterinary surgeon for a number of years, and this scrutiny and control is scrupulously enforced after the animal enters the stables. It has not as yet been demonstrated what

kind of horses are best suited for the preparation of the serum, but we prefer large, heavy animals of middle age. We begin with very small doses of the diphtheria toxin. About 1. c. c. m. of the toxin is injected subcutaneously. Re-action sets in even after this small amount, the animal eats poorly and the body temperature, measured in the rectum, is slightly elevated. This slight re-action, however, rapidly disappears (in one or two days). Then a somewhat larger injection is given, and the dose is gradually increased, until several litres of the toxin can be used at a dose. Subsidence of the re-action must always be awaited and no further injection is given until all the symptoms have entirely disappeared. What care, what caution and what perseverance is required to properly immunize the animals, only those can fully appreciate who have actually performed the work. Frequently it happens that an animal will succumb to the action of the toxin, which may manifest itself after the influence of the poison seems to have ceased. Occasionally an increased intensity of action occurs and the animal perishes. Obviously, also, there must be provided proper attention, abundant food of good quality and absolute cleanliness.

After 3-6 months, when we believe the animal to be sufficiently immunized (in Germany, the law requires at least 100 units per c. c.), a specimen is first obtained, then the full amount of blood is drawn. This is permissible about 2 or 3 weeks after the last injection. The neck of the animal is thoroughly cleansed with soap and water, alcohol, ether, etc., a space sufficiently large is shaved and the large vein of the neck is tapped by means of a canula, or a trocar is inserted. When the full amount is to be drawn, about 4 or 5 litres of blood are allowed to flow into tall, sterilized cylinders. These are placed in an ice chest and allowed to stand 3 or 4 days. The slight wound in the horse heals very rapidly, and after a few days

a further injection of toxin may be given cautiously, in order to extend the immunization.

After 3 or 4 days, the blood in the cylinders has separated into a lower stratum of red blood clot, and above this the clear, yellow serum. The latter is taken off with a sterile pipette and to it is added $\frac{1}{2}$ per cent. carbolic acid. This throws down a small amount of albumen which makes the serum slightly murky; an alteration which is, however, in no wise objectionable, whilst the carbolic acid, on the other hand, acts as a preservative.

3. *Tests of the Serum.*—The most important step now, is to determine the strength of the serum. In order to have a standard for the German Empire and a faultless test, every serum is re-tested in the Prussian Institute for the Investigation and Testing of Serum, at Berlin. Only when this test is absolutely satisfactory, is the serum admitted for sale. As proof of satisfactory test there is affixed to each vial a lead seal, which bears on one side the Prussian eagle, on the reverse, the number of immunity units.

For test purposes, the laboratory at Berlin furnishes us with their toxin, so that our processes may be in harmony with theirs. The principle of the test is as follows: It is ascertained what is the least amount of serum requisite to perfectly protect a guinea pig against a surely fatal dose of the toxin and without even the occurrence of a localized re-action at the site of the injection. As a basis it has been accepted that if 1. c. c. of serum is sufficient to obtain this result, the serum contains 1 immunizing unit in the c. c., if .01 c. c. suffice it has 10 units, with .001 c. c. 100 units per c. c. In Germany, the least allowed by law is 100 units. With a 200 fold serum, .005 c. c. is sufficient. The practical test of the serum may be made in two different ways. The old method is as follows: Assuming that the minimum fatal dose of the toxin had been found to be 1. c. c. for a guinea pig

weighing 500 grains, ten times this amount of toxin was taken (1. c. c. in this case) to this was added .0001 c. c. of serum and the mixture injected subcutaneously in a guinea pig. If the animal remained alive, the serum had at least 100 units to the c. c., and further tests were made with smaller doses of the serum. To-day, we test differently. We determine how small a dose of toxin is required to cause a local re-action at the point of injection. This re-action shows itself as an infiltration which is readily perceptible to the touch as a firm, had cord. Ten times this dose is then taken, mixed with serum and injected as before. There should occur no signs of infiltration, nor formation of cords. For these tests the government laboratory furnishes the toxin, the same as used in their own tests, together with the required dosage. Having determined the strength of the serum, further tests are made. Purity and absence of germs is determined by inoculating a small quantity of the serum on a culture medium. No germs should be present if the serum is to be admitted for use.

Finally, the harmlessness of the serum is tested. A relatively large dose of the pure serum is injected into a mouse and must cause no harmful effects whatever. On completion of all these tests the controlling official, who has been present throughout and is a health officer, seals a number of sample vials which are sent to the government laboratory at Berlin to be re-tested. If this proves satisfactory, the serum is recognized as answering the requirements and it may be drawn off into vials, but only under official control. As evidence of the potency and purity of the serum, a lead seal, as already stated, is affixed to each vial. After six months, the Berlin officials again test the serum, and if it is found to have at all deteriorated, the control number is cancelled and the serum exchanged for a fresh preparation.

The views as to the action of the serum are still

somewhat at variance. No reasonable doubt as to its efficacy, however, can exist when one sees how even .0001 c. c. suffices to save an animal which would surely have died within a few hours without this milligram of serum. Experiments on animals may perhaps not be accepted as equalling the conditions in man, yet many unquestionable and favorable results in human patients must be ascribed to the action of the serum. This alone might justify us in considering that the laboratory tests on animals as to the efficacy of the serum fully represent similar conditions in man.

We, Dr. A. Ruete and myself, devoted much time and pains to our preparatory work in producing the serum before we succeeded in obtaining a product which would conform to the legal requirements and be admitted to use by the Berlin officials. We have arranged for preparation of the serum on a large scale and it gives us much pleasure to be enabled to say that the serum which is known as Anti-diphtheritic Serum (Ruete-Enoch), has found great favor both in the large hospitals and in private practice.

